

Figure 1 : Isolation of monokaryotic strain deficient in laccase activity.

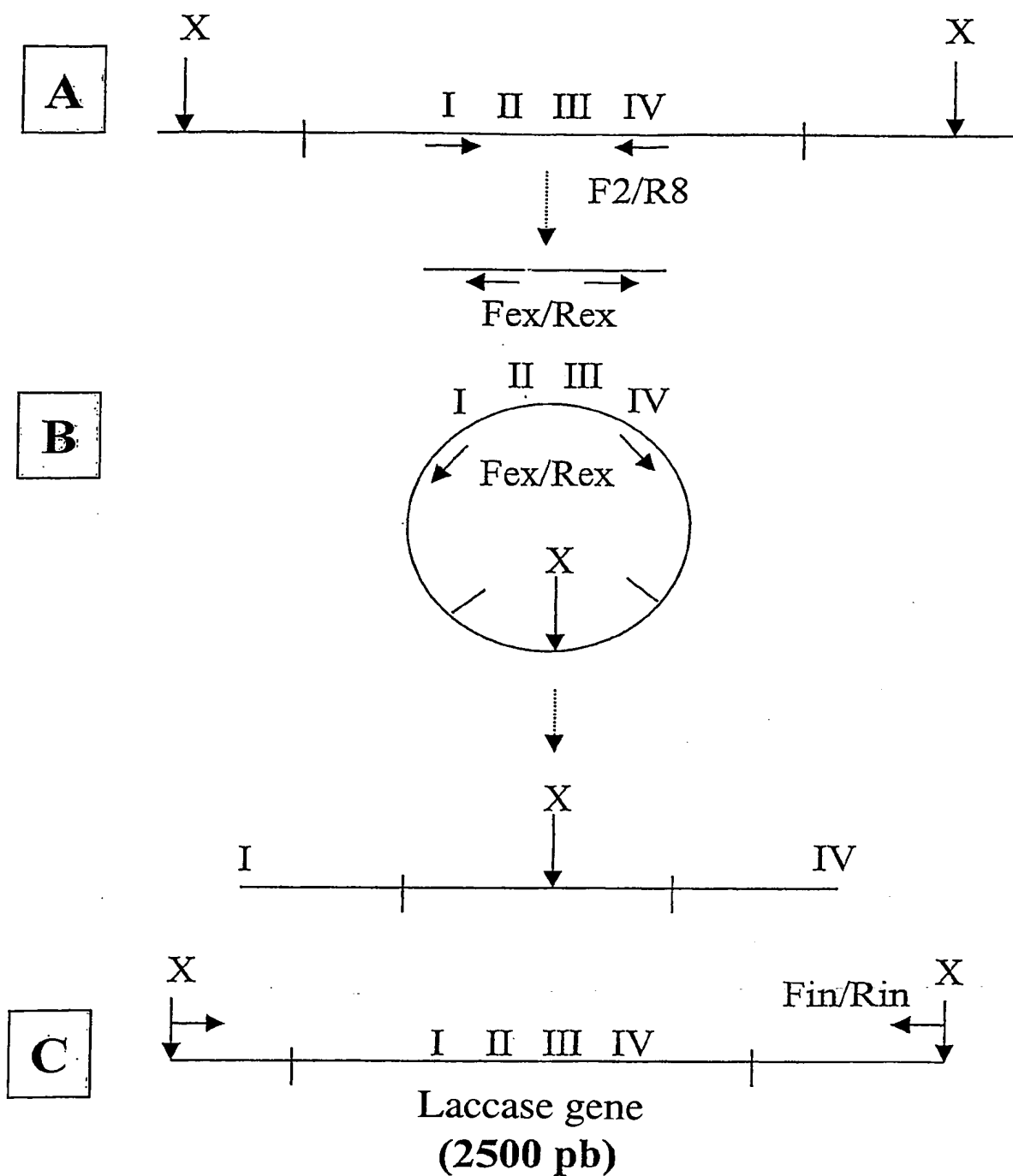


Figure 2 : Isolation of the gene coding for the laccase of *Pycnoporus cinnabarinus* laccase.

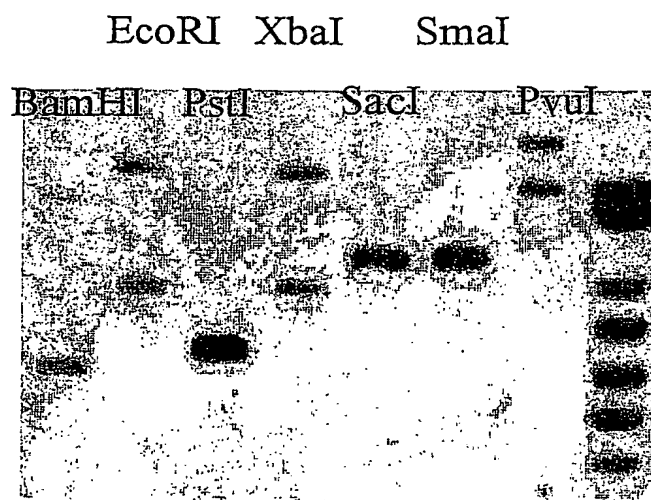


Figure 3 : Southern blot study of the gene coding for the laccase of *Pycnoporus cinnabarinus*.

CTGCAGACATCTGGAGCGCCTGTCTTTCCCTAGTATAAATGATGCTGTCCGAGGTCCTTGAAGACCGCTCGASTCCCACTTGAGTTTATAGGTAGGAC 100
 CTGTCCACCAAAACCCCTCTTTCTGATCATGTGCGAGGTTCCAGTCCCTCTTCTTCTGCTCCTCGTCTCCCTCACCCTGTGGCCACGCGAGCCATAGGGC 200
 M S R F Q S L F F F V L V S L T A V A N A A I G P 25
 CTGTGGCGGACCTGACCCCTTACCARTGCCAGGTGAGCCCGATGGCTTCCGCTCGCGAGGCGCTGCTGGTGAACGGTATCACCCCTGCCCTCTCATCAC 300
 V A D L T L T N A Q V S P D G F A R E A V V V N G I T P A P L I T 58
 AGGCAATAAGgtatgtatatgtcgtcgtccctcagagctacatacatctgatccacaatcgtttagggcgatccgattccagctcaatgtcatccgacag 400
 G N K G D R F Q L N V I D Q 72

F2

TTGACAAATCATACCATGTTGAAACATCTAGTATTgtaagggttcagtttttcccgactaccatgttattgaccatcaccactcgtag CATTGGCACGG 500
 L T N H T M L K T S S I R W H G 86

(I)

CTTCTTCCAGCAAGGCACGAACCTGGGCGGATGGTCCCGCTTCTGTAACCACTGTCCTCATCGCTTCCGGCCACTCGTT CTTGTATGACTTTCAAGTTCCC 600
 F F Q Q G T N W A D G P A F V N Q C P I A S G H S F L Y D F Q V P 121

(II)

GACCAAGCAGgtacgaattccgtacacgttttcattgctgcgaactaaacctctcttactaggGACTTTCTGGTACCATAGCCATCTCTCCACGCAATA 700
 D Q A G T F W Y H S H L S T Q Y 137

(II)

CTGCGATGTTTGGAGGGGCTTTGCTCGTCTACGACCCCAACGATCCTCACGCTAGCCTGTATGACATTGATAACGgtgagcagatcatggtatcgcaa 800
 C D G L R G P F V V Y D P N D P H A S L Y D I D N D 163
 tattgctccacttatgcttccctggcatccagACGACACTGTCTATTACGCTGGCTGATTGGTATCACGTTGCTGCCAAGCTCGGACCTCGCTTCCCgtac 900
 D T V I T L A D W Y H V A A K L G P R F P 184
 gtgtcaaatgtctacgagagatctcacatatacagactagactcacttcgctgattacagATTGGCTCCGATTCAACCCCTATCAATGGACTTGGTCGAA 1000
 F G S D S T L I N G L G R T 198

CCACTGGCATAGCACCGTCCGACTTGGCAGTTATCAAGGTCACGCAGGGCAAGCGgtaatgtggtggtcatcactgcacattggtctctgatacatggtc 1100
 T G I A P S D L A V I R V T Q G K R 216
 ctgtttccacagCTACCGCTTCCGCTTGGGTGCTGCTTCTTGCATCCGACCATACATTACGACTTGATATACACAAATGACTATAATTGAGGCGGA 1200
 Y R F R L V S L S C D P N H T F S I D N H T M T I I E A D 245
 CTCGATCAACACTCAACCCCTAGAGGTTGATTCAATCCAGATTTTTCGCGCGCAGCGCTACTCCTTCGTGgtagg tctaggctcctgtcatcaagtttg 1300
 S I N T Q P L E V D S I Q I F A A Q R Y S F V 268
 cagacattcttagatcacaccttttcaatgcagCTGGATGCTAGCCAGCGGTGGATAACTACTGGATCCGCGCAACCCCTGCCTTCGGAACACAGGTT 1400
 L D A S Q P V D N Y W I R A N P A F G N T G F 291
 TTGCTGGTGAATCAATTTCTGCCATCTGCGTTATGATGGCGCACCGAGATCGAGCCCTACGTCTGTCCAGACTACTCCTACGAAGCCTTGAACGAGGT 1500
 A G G I N S A I L R Y D G A P E I E P T S V Q T T P T K P L N E V 324
 CEACTGCACTCTCTCTCGCCTATGCTGTGgtacgtgtctcaaagaacctcgatcactaagtgcagtgcacatcatatggtgcagtgcagCCTGGCAGC 1600
 D L H P L S P M P V P G S 337
 CCGGAGCCCGGAGGTGTCGACAAAGCCTTGAACCTTGGTCTTCAACTTCgtgagtactggtgcgctccgttagcacaggttcgaacaagcctgataccat 1700
 P E P G G V D K P L N L V F N F 353
 gcagAACGGCACCAACTTCTTCATCAACGACCCACCTTTGTCCCGCGCTGTGCTCCAGTCTTGCTACAAATCCTCAGTGGGGCGCAGGCGGCTCAGGAC 1800
 N G T N F F I N D H T F V P P S V P V L L Q I L S G A Q A A Q D 385
 CTGGTCCCGGAGGCGAGCGTGTTCGTTCTTCCGACCACTCGTCCATTGACCATATCCTT CCCTGCCACTGCCAATGCCCTGGATTCCCCCATCCGTTCC 1900
 L V P E G S V F V L P S N S S I E I S F P A T A N A P G F P R P F H 419

(III)

ACTTGACCGTgtacgtctgccttccctcgtctaaggcggagtcgatatctgactcccatcacagCACGCTTCTCGTGTGCTCCGGAGCGCC GGGAGC 2000
 L H G H A F A V V R S A G S 433

(III)

AGCGTCTACAACTACGACAACCGATCTTCCGCGACSTCGTCAGCACCGGCGAGCCCGGCGACA ACGTCACGATTGCTTCCGAGACCAATAACCCAGGCC 2100
 S V Y N Y D N P I F R D V V S T G Q P G D N V T I R F E T N N P G P 467

R8

CGTGGTTCTCCACTGCCACATTGACTTCCACCTCGACGAGGCTTTGCTGTAGTATGCGCCGAGGACACTCCGGACACCAGGCGCGGAAC CCTGTTC 2200
 W E L H C H I D F H L D A G F A V V M A E D T P D T K A A N P V P 500

(IV)

TCAGGCGTGGTGGGACTTGTGCCCATCTATGATGCACTTGACCCAGCGACCTCTGAGCGGGATGTTACTGTGACCTGGT GTGGGGGGAACATGTCGA 2300
 Q A W S D L C P I Y D A L D P S D L 518
 GGGCTTTCATCGATCAGGACTTCAAGGTTGGCATATATACCTACGCGCTGGATGACTCGGACAGCGTGTGGGCGTGGGTGTAACCTGCTGATGT 2400
 TGAATAAAGGATTTTATGTAGAACAATTTATGAGCAATCAGCAATCAATAGGATTGTGTGCGTTTCGACGAATGTCTTGTCTCCCTGACATTACTTTTG 2500
 TGCGAGAATGGGTCCATGATACACATCATTTAGCTCTCAATACCAAGAAGGATTACCCATGTCAATACCAAGATCATGTCTTCGCTGTCCGCAATGG 2600
 TCTCATGTTCGTTGAGCAGATCGCAGTACGTTGAAAAGCGATTAGTAT TACATGCAACATGCAACATTGGAAGGGGGCATGACAGAGGTTACGCTCGCG 2700

TCAGTCCGCCAAGTAGCGACCTTTGCCGCACTGCTGTTAACTGAACGTATGCTTCAAGTCCGTCGGTATCGAGAGCGATCGTGTACGTTCCGGGAT 2800

AGATCCCATGATCCCCGCTCTGGTTCGGTGGGCGATGGCCCCGAGCGCTTCGCGATCGCGCTTTTCTAGGGGCGAGGCGCTGTACCCG 2900

CGTGTACGAGACGAGCTGCTTGTTCGGGTGGGGCGAAGGCCGGAAGGAGCCACTCACGAAGAGCAATGCGACGTAATCCGAGGTAGCCTTGCCCGTGTTA 3000

GTCACACGCACGGAGAAGCTGTGAGCGGCGCGAGGTCGAGGAAGGCGCGCTCTTCTGACCGCGCTGTACGAGGTCGGAATCGAATACGTCGATGGCG 3100

GTCTCTCAAAGTCCGTGACGTTGGTGCATCGGCGCGCGCGCTGGAGCTGCCAAGAGAAATCGAAGGTGGTGAAGTGCAGTCCAAGGCCAATTCGTA 3200

GACCGCGGTGCCGGTGTACCACTTGTATGTACGCCCCGGGTTCGACGCGCTTGGGCGAAGGGT CATGTAGTCATCGGAACCTGATCAGCGTAGATGGCT 3300

GGGTATTGGGTGATGGGCGAGGCGCTCTGCAG 3331

Figure 4 : Sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus*

AGATCTCCGAACCAGAAATGCGATTGCGTTTCAGGCCCAATTAAGAATAAAGCTGCGTCAGGGCAGCGACGTA
 TCTTGATCCATCATTGACTCACCGGCATCGGCGTCAACACCAAAGCAAGCTCGTCCCACCCATAGGCGTGCA
 CCGGCCGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCG
 CGGCGATGAAACGTTTCCCACCATTTGGGAAGAAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGC
 GCGCTCGCGCCTTTGCCGAGAGGCCGGCGGGCGACATGCACAGCGAAGGTCCGTTGCGGATGGGAAGCAGG
 CAATCAGTGGGTGTCCTACGCCGCCACGATGGTGGGGAGCGTAGGCGCCCTCCCATAAGGCGGCAAGCATC
 ATGATGCTCTCCGATTTCGGGAAGCCTGGTGGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC
 GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACC
 ATCGGCATCCTCAGCCTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGC
 TTCTTGGATGATCATGTGCTAACTTTTTCTGACCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGT
 ATGCCTCCCATTTCATAAACGATAACCCCTTCTTTAGGTTGGTTCATCTCCATAGAGCGGCACGCTCTCAAGG
 CCTAGGCTATTACACCTCCTTCGCAACATCCCTATTACGGTGTCTGTAAGGAACGACTTGTTCATGGGATC
 ACATGAAGTGCAGCATACTGTTCCGCCGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGT
 TCAGTACCACATGGCAAAAAAGCTGCACCATACTCTTTATGGTGGTGTTCGTCGAGTGGTATACAGTCAT
 TCATGAGGGAATGCCCACCGGATAGGGTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGT
 CTTTGTTCATGAATATCATGGGTACATGTGGAGACGGTTAAACAGCGTTGACTGTGAATCCCTGGTGTGT
 GTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCAGTTCTTTGCGAGCGGCACAG
 GCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGG
 AGCGAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCTCTCTCACCATTGGGAAGAT
 GTGAAAGGCTCCATCATATAGCGGCTCAACTCTACCTCGAATGTCCAAACACGGCGGGAATACTTATTTATG
 TGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAAGTTGGTAAGTCCCGCAATCTGCGGTTTCAGGCAACAGT
 CTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAATGCGCACACACGGAGGCTTTA
 GGAGATGAAGCGCCCGTGAGCGGTAAAGGAGTTGGTTACCGCCGCCCCGACCGACTCTCTCTCTTTCCAG
 CATCATGTCTCGGCGCAAACCTTTACCCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGCT
 CTCATGACGTCCGCAATCCAGACCCCTAGCCGGTTGCTTACTCATCGTTATCCCTGCCGCCATGGTAGTGGA
 GTCAGCCTGGCCAGTGCCTAGTCCCGTCTCTTGTGCTGACTAGAGAAGCCCCATGAGACAGCGTTTTTTGC
 TTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTGCACGCCCAGAGGTATTGGGCTCGTCA
 GATTCACGTTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAATGCTATAGCCCTT
 CATAGCCCGCTATGAGAGTCGCAAAAGGCTTGTAGTCAGGTGCGTTCGAGTGGCTCTCACGAAGAGCGTCAA
 CTTCCGCGACAGCCGCTTTTCAGGGCAAGATAGATCCTCCATCATCCCTACTGCGCTCAGCGCCGGTAC
 CGAACAATTGACTTACCGACATCCTCCGGGACGCGCAAATGCTGTTGACGGAACGTAATCCTCTTCGTCCC
 GCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTTCGCGCGACGGCCGCTCATCAGGACCAGACAGTCTCAAT
 GTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTGGTGCACCGTCGCTTAC
 GCTGACCTTCGGGATACTGTCTGACAGATCTGGAGCGCCTGTCTTTCCCTAGTATAAATGATGTCTGTC
 CGCAGGTCCCTGAAGACCGCTCGAGTCCCACTTGAGTTTTAGGTAGGACCTGTCCACCAAACCCCTCTTTCT
 GATCATG

Figure 5 : Sequence of the promoter sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus* (up to the ATG coding for the methionine of the laccase).

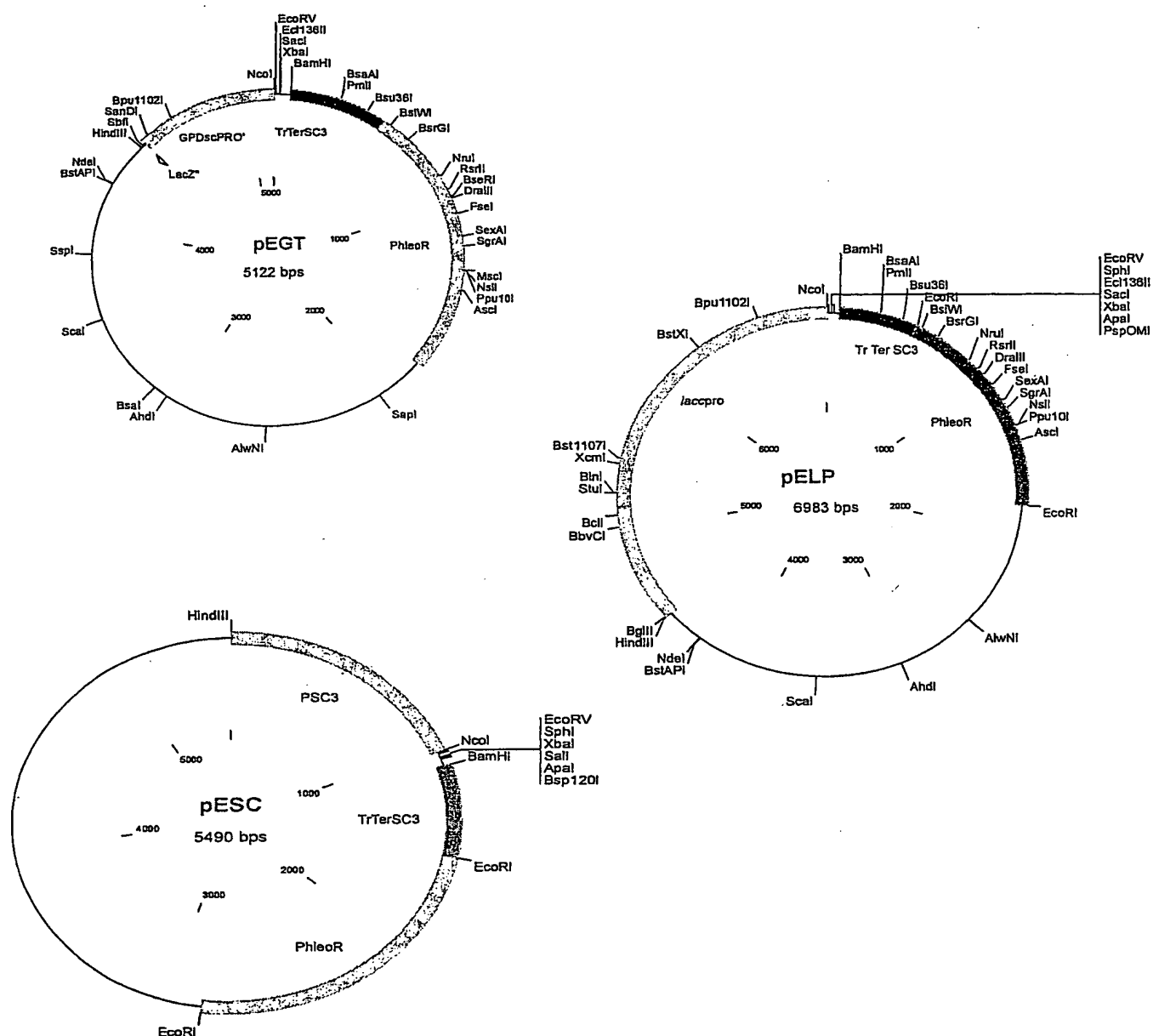


Figure 6 : Restriction map of the three expression vectors used for the production of laccase in *Pycnoporus cinnabarinus*.

CATGGGATATCGCATGCCTGCGAGCTCTAGAGTCGACGGGCGGCTACCGCGGCCGCTTAAGACGCGTGGATCCGACAGGTGAAC
 GCGCCTATCGGTGGGATATTGCGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTACTGCCTAGCAAATTCGGAATCCCTTCGATGT
 CATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAGGTGTTGACTCATTCCTCGATAAATGAACATTGTTGTTGTTG
 TTCTCTATCCGCTCAGTCACGCGACCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAG
 TAAAGGCTGAGTCGTGACTAAAGCACTCCACTTTACGGCGAGGATGCCAGTCTACGTATGAATGAAGCCTCAGTCCCGAAGTAA
 GGGGTACAAAAGGAGGGTGAAGGTGGACGTTTTCTTACCATCTTCCACCTCCAGACCACATGCCGGGAATTCAGCTTGCT
 CAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATC
 ATTTTGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCTTTCTTTTACTCTCCGGCCAGTCTATGTAGAGGTAAA
 GTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCGCTTGTCTTCGCCGCTTAGAGCGCGCGCTCTGCTTCGCCGCGTAGACG
 AGCAGTTCGACAGACCGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTTCACGAAGCTCTTGCTGATCGCGATGCCG
 GGGATCGATCCACGCTCTTAAGGCGCGCGGTACCCCTCGGACCCGTCGGCGCGCTCGGACCGCGGTGTTGGTCGGCGTCGG
 TCAGTCTGCTCCTCGGCCACGAAGTGCACGCGAGTTCGCCGGCGGGTCCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGAT
 CTCGGTCAATGCCCGGCCGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCGCGCGAC
 CCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTCACGTCTGCCCGGACCAACCGGC
 GAACTCGTCTCCACGGAATCCCGGAGAACCCGAGCTCGAGAACTGACCGCTCCGCGCGCGGTGAGCA
 CCGGAACGGCACTGGTCAACTTGGCCATGCATGGTGTATGGGCATTATGTGTGATGGGATGCGATGGGAGAGGGGAAGTGTCTGGATG
 GGAGTGTGGAGAAAGAGGGAGACGGCGGGCGGCGCGCTTTTATACCCACGCCGAAAGATCCGATCGATACTGACAAAACGGGA
 TGAACACATCGCGCGCGCTGGACTGCGCGCCATCTGCAAAATGCCAGCGCAGTCCCGTCCGGCGCCACCACAGCCCTGGTTCGAGT
 CCCCTCGAGGCGACGCTCTTATCTATCCATCGCGCAATTCGAGGTCGAGAAACAGTCTCTTCGCAAGAGCTTCTTCGCACT
 TGGGCTGCGACCTGTCTACCTCTCATCTTAACCCCTCCGCGGCTTCGCACTACAGTTACTAATCTCACACCGAAGGCTCTCGCGC
 CACCCTCCGATCCCGAGCACGTTCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCGGGAATTCGT
 AATCATGGTCATAGCTGTTCTCTGTGTGAAATTTGTTATCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAAG
 CTTGGGTGCTTAATGAGTACGTAACATTAATGGCTGTGCTCTACTGCCGCTTCCAGTCGGGAAACCTGTCTGTCGACGCT
 GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGTATTTGGCGCTTCTCCGCTTCTCTGCTACTGATGCTGCTGCTC
 GTCGTTCCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA
 CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTGTGGCGTTTTTCCATAGGCTCCGCCCGCTGACG
 AGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAAGGCGTTTCCCGCTGGAAGCTCC
 CTCGTGCGCTCTCTGTTCGACGCTGCGCTTACCGGATACGCTTCCGCTTCTCCCTTCGGGAAGCGTGCGCTTCTCATAGCTC
 ACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTACGCCCCAGCGCTGCGCC
 TTATCCGGTAACATATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA
 GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTTAACACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCT
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 GCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAA
 AACTCACGTTAAGGGATTTTGGTTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAA
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 ATCCATAGTTGCTGACTCCCGTCTGTGATAGATAACGATACGAGAGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG
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 TGTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTGGCCGAGTGTATCACTCATGGTTATGGC
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 TGTATGCGGCGACCGAGTTGCTCTTGCCCGCGCTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATT
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 TCTTCAGCATCTTTTACTTTCACAGCGTTTCTGGGTGAGCAAAAAAGGAAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGAC
 ACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTTGAAGCATTATTCAGGGTTATTGTCTCATGAGCGGATACATATTG
 AATGTATTTAGAAAAATAAAATAAGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTTATTATCA
 TGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTTCGTCTCGCGCTTTCGGTGTATGACGGTGAAAAACCTCTGACACATGC
 AGTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCGTCAGGGCGCGTCAGCGGGTGTGGCGGG
 TGTGCGGGCTGGCTTAACATAGCGGCATCAGAGCAATTGATCAGAGTGCACCATATGCGGTGTGAATACCGCATCAGATGCGCTA
 AGGAGAAAAATACCGCATCAGGCGCCATTTCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCCGTTGCGGGCTCTTCGCTATTA
 CGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAAACGAC
 GGCCAGTGCCTAAGCTTCATGCCTGCAGGTCGACGACCGAGCGCGGCCACCCAGCCTATCCCGCGCGGGTTCGGGACCCAAAAATAA
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 GAACACCATCGCGGACGGCCAGTGCTCTGGDCAGCTGAGCGTGCATTGTTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC
 GGGATCGGAGGGTGGCGCGAGAGCTCTTCGGTGTGAGATTAGTAAGTGTACTGCGAAGCCGCGGAGGGGTAGGATGAGAGGTAG
 ACAGGGTTCGACGCCAGGTGCGAAGGAGTGCAGAGGACTGTTCTTCGACCGCGCACCTGCAATTGCGCGCATGGATAGAAATAGA
 CGCTGCCCTCGAGGAGTGCAGGAGGCTGGTGGTGGCGCCGACGGGACTGGCTGGGCAATTGTCAGATGGCGCGCAGTCCAG
 GCCGCGCGCATGTTGTTTATCCCGTTTTGTCAGTATCGATCGGATCTTTCGGCGTGGGTATAAAAGCGCGCGCGCTCTCCCT
 CTTTCTCCAGCACTCCCATCCAGAGCACTTCCCTCTCCCATCGCATCCCATCACACAATAATGCCCATCAC

Figure 7 : Nucleotide sequence of the vector pEGT, containing the *gpd* gene promoter (4480-5112), a phleomycin resistance marker (507-1822) and the *sc3* gene terminator (71-507).

TTTCCCAGTCA'CGACGTTGTAAACGACGGCCAGTGCCA

Figure 8 : Nucleotide sequence of the vector pESC, containing the sc3 gene promoter (1-1033), a phleomycin resistance marker (1540-2855) and the sc3 gene terminator (1104-1540).

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCGCGGTACCGCGGCGCCCTTAAGACCGCTGGATCCGCAGGTGAACGCGC
 CTATCGGTGGGATATTGGGGGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAAATTCGGAATCCCTTCGATGTCATAGGGT
 CGCGGACAAGTGATCGTCTTGCTACATACTCCAAGGTGTGACTCAATCCCTCGATAATGAACATTGTTGTTGTTGTTTCTCTATCCGC
 TCAGTACGCGACCCACACGTGCATGGTTGAATTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGT
 GGAATAAGCACTCCACTTTACGGCGAGGATGCCAGTCTACGTCAATGAATGAAGCCTCAGGTCCCGAAGTAAGGGGGTACAAAAGGAGG
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 ACTCGTTGTGCTACAGGCAATGAGCTTACGAAAGCTCTTGCTGATCGCGATGCGGGGATCGATCCACGCGTCTTAAGGCGGCGCGGT
 ACCCCCTCGGACCCGTCGGGCGCGCTCGGACCGGCGGTGTGGTCCGCGTCAGTCTGCTCCTCGGCCAGAAAGTGACACGAGTTG
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 ACCGCGCTGATGAACAGGGTCAAGTCTGTCGGGACCAACCGGCGAAGTCTGCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGGT
 CAGAACTCGACGCTCCGGCGACGTGCGCGCGGTGAGCAGCGGAACGGCACTGGTCAACTTGGCCATGCAATGGTGAACGAGACCGCGGCGCGCTTTTATACCCAG
 TGATGGATCGCATCGGTGAGGGAAGTGCTCTGGATGGGAGTCTGAGAAAGGCAAGTGGCAGACCGCGGCGCGCTTTTATACCCAG
 CCCGAAAGATCCGATCGATACTGACAAAACGGGATGAACACATCGCGCGCGCGCTGGACTGCGCGCCATCTGCAAAATGCCAGCCAGTC
 CCGTCCGGGCGCCACCCAGCCCTGGTTCGAGTCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGACAGGTGCGCGGTGCA
 AGAACAGTCTTCGAGTCTTCTCGCACCTGGGCTGCGACCTGTCTACCTCTCATCTTAACCCCTCCGCGGCTTCGACAGTACAGTTACTA
 ATCTCACAGGAAGAGTCTCGGCTGCGCGCAACCCGATCCGAGCACGTTCTTCAAGAAAGTGGCAAGTGGCAATGCAATGACGATGCA
 ARATCAGATCCCGGGAATTGTAATCATGGTCAATGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATACGAGCC
 GGAAGCATAAAGTGTAAGCCTGGGGTGCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCCAGTCGGGA
 AACCTGTGTCGACGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGGCGCTCTCCGCTTCTCGTCACTG
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 AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTCAATCCATAGTTGCCTGACTCCCCG
 TCGTGTAGATAAATACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATT
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 CCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTTCTTACTGTCAATGCCATCCGTAA
 GATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTCTGAGAAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGCGCTCAATACG
 GGATAATACCGCGCCACTAGCAACTTTAAAGTGTCTAAGTATGCTTAAATTAAGGTTCTTCGGGGCGAAAACTTCAAGGATCTTACCGCT
 GTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTGATCTTACGATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACA
 GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCACTCTTCTTTTCAATATTATTGAAGCATT
 TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAAG
 TGCCACCTGACGTCTAAGAAACCATATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTCGTCGCGCGTTTCGG
 TGATGACGGTGAAAAACCTCTGACACATGCAGTCCCGGAGACGGTCAAGCTTGTCTGTAAGCGGATGCGGGGAGCAGACAAGCCCGTCA
 GGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAATACTATGCGGCATCAGAGCAGATTGTAAGTGAAGTGCACCATATGCGGTG
 TGAATATCCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCCATTGCGCAATCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGT
 GCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGAATGTGCTGCAAGGCGATTAAGTTGGGTAAGCCAGGGTTTCCAGTCAAG
 ACCTTGTAAAAACGACGGCCAGTGCCCAAGCTTAGATCTCCGAACAGAAATGCGATTGCGTTACGGCCCAATTAAGAAATAAAGCTGCGTCA
 GGGCAGCGACGTATCTTGATCCATCATTGACTCACCGGCATCGGCGTCAACACCAAGCAAGCTCGTCCACCCATAGGCGTGCACCGGC
 CGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGCTGAGACGCGCGCGGATGAAACGTTTCCACCA
 TTGGGAAGAAACGTTCTGCGGCCATCATCCCTTACCGGATGACAAGGCGGCGTCCGCGCTTTGCCGAGAGGCCGGCGGCGACATGCA

Figure 9 : Nucleotide sequence of the vector pELP, containing the laccase gene (promoter 4457-6983) , a phleomycin resistance marker (507-1822) and the sc3 gene terminator (71-507) (continuation of the sequence on the following page).

CAGCGAAGGTCCGTTGCGGATGGGAAGCAGGCAATCAGTGGGTGTCCTACGCCGCCACGATGGTTCGGGGAGCGTAGGGGCCCTCCCA
TAAGGCGGCAAGCATCATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC
GTTCTTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCGAGGAACCATCGGCATCCTCAGC
CTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCTAGATGTGAGCGGGCTTCTTGGATGATCATGTGCGTAACCTTTTCTGA
CCTCGTCCGGTGGTACGCATGGCAGGATTGAGCATTACGGTATGCCTCCCATTCATAAACGATAACCCCTTCCTTCAGGTTGGTTCATCTC
CATAGAGCGGCACGCTCTCAAGGCCTAGGCTATTACACCTCCTTCGCAACATCCCTATTACCGGTGTCTGTAAGGAACGACTTGTCAAT
GGGATCACATGAAGTGCAGCATACTGTTCCGCCGCTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGTTCAGTCACCA
CATGGCAAAAAAGCTGCACCACTCTTTATGGTGAGTTGTTCTGAGTGGTATACAGTCATTATGAGGGAATGCCACCGGATAGG
GTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGTCCTTGTTCAATGAATATCATGGGTACATGTGGAGACGGTTAA
ACAGCGTTGACTGTGAATCCCTGGTGTGTGTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCAGTTCCTTG
CGAGCGGCACAGGCAGGCATCGCGCAACAGATCCCGAGCCATCCGCGCTTGACATTGGGGATACCTGAAGCCCTTCAGGTACGGAGC
GAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCTCTCTCACCATTGGGAAGATGTGAAAGGCTCCATCATAT
AGCGGCTCAACTTACCTCGAATGTCCAAACACGGCGGGGAATACTTATTTATGTGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAA
GTTGGTAAGTCCCGCAATCTGCGGTTTCAGGCAACAGTCTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAA
TGCGCACACACGGAGGCTTTAGGAGATGAAGCGCCCGTGAGCGGTAAGGGAGTTGGTTACCGCCGCCCGACCGACTCTCTCTTT
CCCAGCATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCTGTCTCTCATGACGTCC
GCAATCCAGACCCCTTAGCCGGTTTCGTTACTCATCGTTATCCCTGCCGCCATCGTAGTGAGTCAAGCTGGCCAGTGGCTAGTCCCGTCT
CTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTGTCTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTG
CACGCCCAGAGGTAATTGGGCTCGTCAGATTCCCAAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAAATCGGCCGGAATGCT
ATAGCCCTTCATAGCCCGCTATGAGAGTCGCAAAAGGCTTGTCAGTCAGGTCCGTGCGTGGCTCTCACGAAGAGCGTCAACTTCGCG
CGACAGCCGCTTTTCAAGGCAAGATAGATCCTCCCATCATCCCTACTCGCTCAGCGCCGGTACCGAACAATTGACTTACCGACATC
CTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCGCCGCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGA
CGGCCGCTCATCAGGACCAGACCAAGTCTCAATGTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTG
GTGCACCGTTCGCTTACGCTGACCTTCGGGATACTGTCTGACAGACATCTGGAGCGCCTGTCTTTCCCTAGTATAAATGATGTCTGTCC
CGAGGTCTTGAAGACCGCTCGAGTCCCACTTGAGTTTTAGGTAGGACCTGTTCTCCACAACCCCTCTTTC

Figure 9 : Nucleotide sequence of the vector pELP (continuation), containing the laccase gene (promoter4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene terminator (71-507).

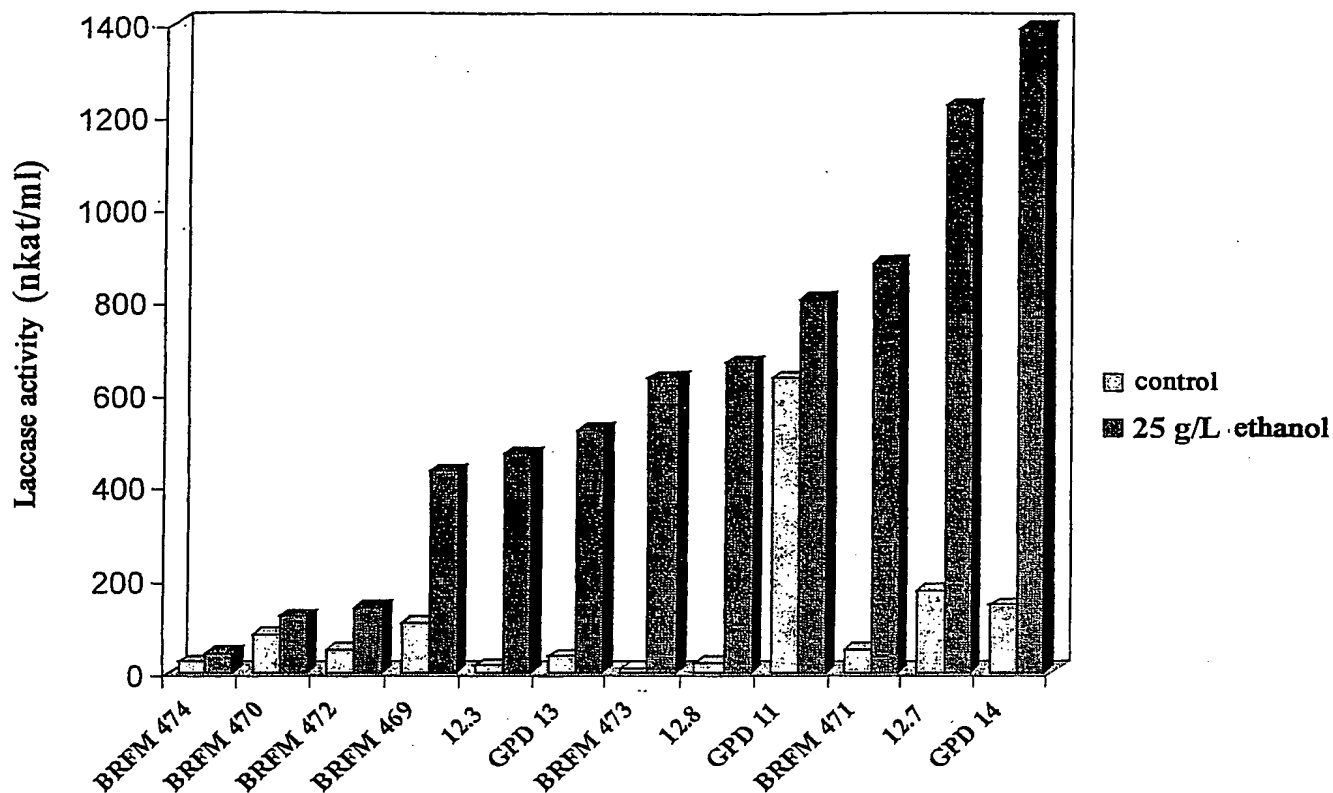


Figure 10 : Results of production of the transformants having the most significant activities. The culture was carried out with or without (control) ethanol.

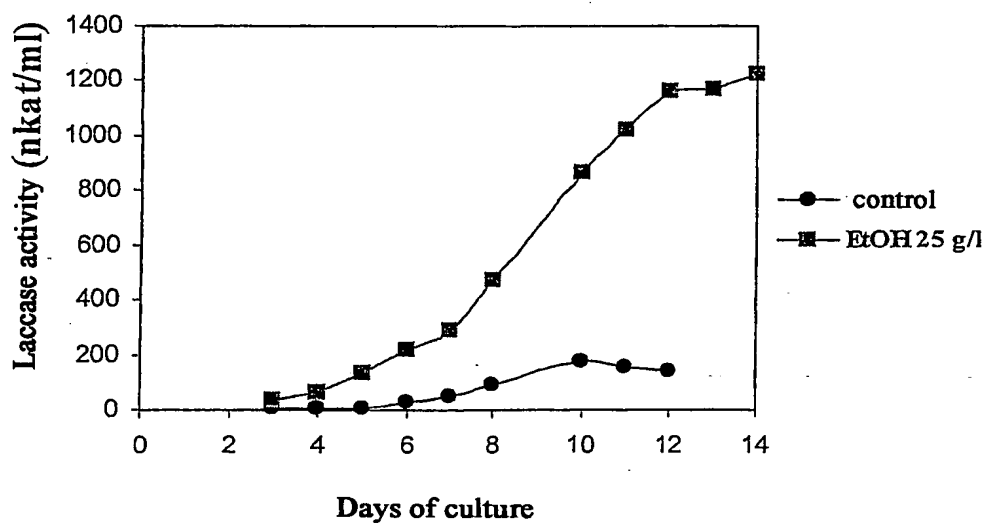
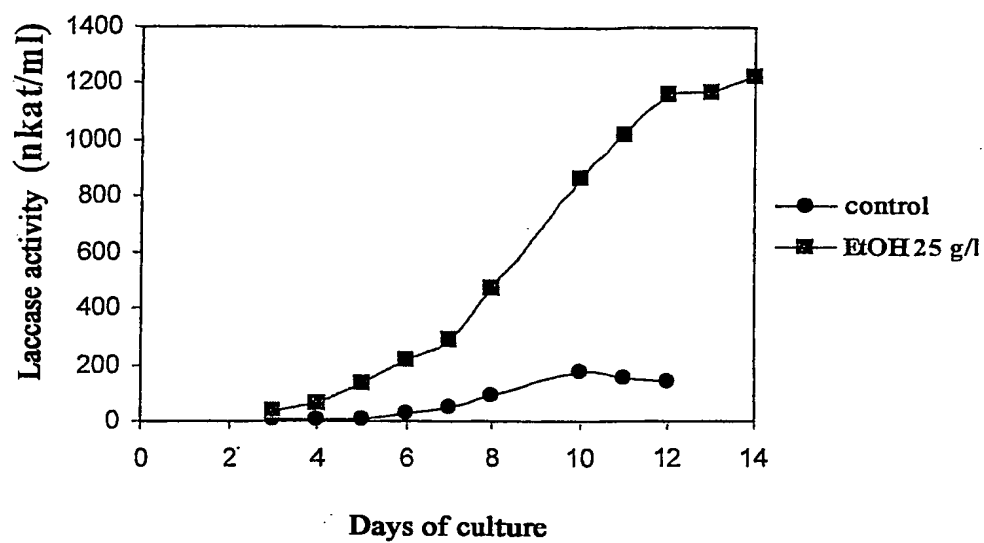


Figure 11 : Monitoring of the laccase activities of the transformants GPD 14 and 12.7 as a function of time with or (control) without ethanol

TGGGGAGATGGTTCTATATATCAAAATGATCTTCTGTCTGAGCTTCTCTGCTCTTGTGTTTCTGCTTGTGAGTGGCGGACATGCTTTTATTAAACCAT 100
 TGGCGAGCTGCCCCGCCCAAGGAGATAGCATAATCGCCTGAGAAACCTAGTCTGTCTCATGGCGGTGTAAACGTTCTTGGGACTTATTTTCGCACTTCTC 200
 TCAAGATATAAAGGCTATTTGTGATACGGTTTCTAATCCCGAGCGTCCCTCGAAGAGTGGGCTGCTCTCACTCTTCGCATTCCTTACTGCTTTAAA 300
 CTCAGTTCATGCCGCTGTGGGTCCTGTTACGGACTTAACACTGATCGTAGATACTGTGCCCCGACGGTGTCTTTTCGCGCGGGAGGGTGAAGCTTTG 400
 S V H A A V G P V T D L T L I V D T V A P D G A A F A R E 43
 CGACTGTAAATGCCGGATTTGAGTTTCTAATTATAATCTTCCAGGCCATGTGCGTCCAAGAGGAACCAAACCTCCGTCATTTGGTCCGGTCATCGTAGGTGGG 500
 L D S P N M R Q S T S I H W H G I P Q G N 60
 TAGCTACGAGTCTTCTCCTTCAATTAGCTCATCACCAAGTGATATGATATTAATTAAGGTCAAAGGGGGGACAACTTTTCGGCTCAATGTTATCAACAAT 600
 TTGGATTCTCCGAACATGCGCAATCTACTTCCATTGATGTCATGGCATCTTCCAAGGAAACGGTAGCTGGTATATCGGATATCTATCTGTATCCATT 700
 GACTCGAATATAGGTGAGAAATGGGCTGGTGGCTTGGCCTTCTGAAGCCTGCTCGAATTTATCTTCTGAATTTTATAGATGGCGCGCATTCGTTAAC 800
 AGSTAAGGAGATGTTCTGCTTCCGTTCCGTTCCCGAGAACTAATTAATCTAGTGCCCAATGCCCGCGGAGGGGACTCGTTCTTGTACGACTTTACCGAACCT 900
 TCCAGACTGGCACATTTTGGTATCATTTCCATTTATCAACTCAATAGTGGACTGGAGGGGAGCATTCGTCGTTCTTCTTCTTCTCATCAAGTCA 1000
 F Q T G T F W Y H S H L S T Q Y C D G L R G A F V 150
 CCGCTTCTTCTCACTTATCTAGATCTACGATCCGCTCGACCTTACCGGTTGCTCTACGATGTGACGACGAGTGCATCTGTGATTACTTGGCGGACTG 1100
 GTACCACAGCTATGCGGAGGACATTTCTAATCGCTAGGAGATTTTCCCAAGATGTCTCTCTGCTCTCTGAATCCATGAACTAGTGACGAGGACACTA 1200
 Y H S Y A E D I L I A A G D T 191
 TCCTCATCAATGGTACGGAAGATTCGCCGAGCGCGGAGCGCAACGGAACGGAACGGAACGGAACGGAACGGAACGGAACGGAACGGAACGGAACGGAAC 1300
 I L I N G H G R F A G A G G T A T E L S V I T V E H G K R 220
 CGGCTTGTAGATGTGCTAATTTGTGATAGCTACCGATTGCGAATTTGCGAATATCGCTTTGTCGACCTTGCTTTGCGGTGAAATCGATGCCATACGAA 1400
 CTTTCGCTTATCGAAGCTGACGGTATTACTACTGTGCTGCTGACGGTGGACTCCTTCAATGTAGGCTTACCTTAGCACTTTCCCACTCTGGATCCTCT 1500
 L R V I E A D G I T T 254
 TATGACTTCCCAAGCATCTTTGTGGGCAACGATATAGTGTCTATCCTCCATGCCAACCAGCCTGTTGGAACTACTGTAAAGCTGCCTAAATGTTGCATGAC 1600
 TGFCCATGATCTCAACCCGCGGAGGATTCGGGCGGCTCCGAACGGCGGTGAGCAATTTCCGCGGTGGGATCGACTCGGCTATTCTCCGTTATGTTGGCGC 1700
 CCCAGAAGAAGAGCCCAACACTAGTGAGGATCTCCATCCGACACACTTCAAGAGCAGGATCTTACCCCGCTGATCCTACCGGCGCGCCAGGATCCAC 1800
 TCCCGTGGGGCGCGGACGTTGTCCACACCGTATCAATGGAGTTTGTGAGTGTGGGAGCTTTTCTGGCCCGCTTTATTAATATATCTGGTAGGATGGC 1900
 S R G A A D V V H T V S M E F 348
 GCAAACTTCAATCTCTCTGGATGGCGTGGCCTTCCAAGCGTGGCTATCTCTTTCAAAGATTTATCTAGCTGACGATTTTGAATGTAGCCGACCA 2000
 TGCCCGTCTTCTGCAAAATTTATCGGGAGCGAGACTGCTAATACCCCTTCTCCCGCGGGATCCTTTATCCCAAGCGTCCGACCAATGACATCGTGGAGCT 2100
 M P V L L Q I L S G A Q T A N T L L P A G S F I Q A S H N D I V E L 391
 CAATTTCCAGCTGTCAACGAGAGCGCTGTGCGTGGGACCGTGGCTCCCATCTTTCTTCCAGCTTGAATTTACGCTCTTTTAGACATCAATCCATCT 2200
 N F P A V N V A A V G G E H P I H L 409
 GTGAGCGCAGCGGGACCTTTGGCTTATGGCATATGACTTATTTATAGCATGGCCATGACATTCGACGTATACGCTCTGCTGGAACGAACTCCGATAACT 2300
 GGTTCATCCGCTATTTTCTTCGACTTCCATAAGATGACGATGGCTCACTATGGTTTTTACCAGCCTCGCAGAGATGTCTATCCACCGGTACCGATC 2400
 W F N P P R R D V V S T G T D 441
 CTAATGACATGTGTACGTGTTTCTGCTATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTTGATTT 2500
 P N D N V T I R F R A D N P 455
 CTGAATCTCTGCTGTCTTGGTTCTCATAATCTCATCTCAGAGGTCCATGGTCTCTCACTGCCACATTTGACTGGCACCTTGAACTCGGCTTTGCTTTGGT 2600
 GATTGACAGAGCGCTAGCGAATGGGACAGCGACATTAACCTCTCTGCTGGCTGCTCTTCTCCCTACACTTGTCTAAGATCGCTCTAGCTG 2700
 I A E A P S E W D S D I N P P A 491
 CGTGGGATGACCTATGCCCTACGTTTGGCTTCTCTTTTACTATTCAAGTTTCTTCACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTT 2800
 A W D D L C P T F A W L L F Y Y F K F P H I L N F T D M M P C R L S 525
 CAGCAGTAAATCGAGTTAAGAACTCAACGTTGACTAAGGAAAGGAAAGCAAGATATGAACTCTCATTTATCTTATATCGACACATTCATATTCAA 2900
 S S N R V K N L N V D 536
 CCTACGGATTTTCTCTCGACCTGAATTCGGTGCTAGATCCCATCTTGGTGGAGTAGGAAAGAAATTTCTTGTATAAACCCATGGGTTCTTCTACC 3000
 ATATATACATAAGCTCGGGGGTGTAGTTAATTCGT 3037

Gene of the laccase of *Halocyphina villosa*

Figure 12